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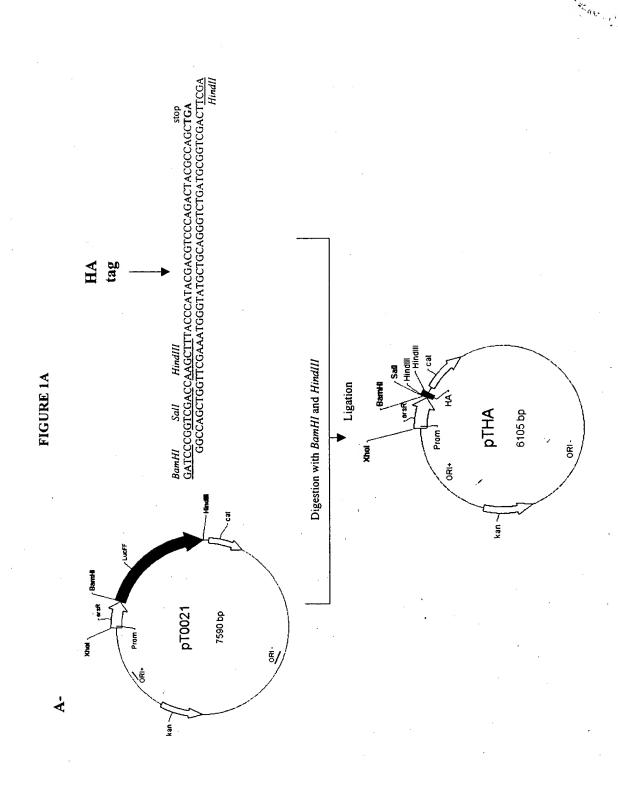
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PCR of pT0021 with XhoF and BamHNR XhoF-5'-AATTCTCGAGTAAAATAACAT-3 Xhol pT0021 7590 bp $AAATCAGGTGACTGTT{\color{red}GA}GAAA{\color{blue}AGGAGGCGGATCC}CG-BamHNR$ Stop of RBS BamHI arsR Digestion with XhoI and BamHI Ligation PCR of pT0021 with LucFFB and LucFFH LucFFB-5'-CGGGATCCATGAGGGGTTCCGAAGACG pT0021 BamHI Start Original BamHI of LucFF was modified 7590 bp Modified between stop GAAAGTCCAAATTGTAAGCTTGGG-LucFFH of arsR to BamHI Stop of HindIII LucFF Digestion with BamHI and HindIII Ligation - Modified in the vicinity of BamHI - Cloning site for ORFs: BamHI and HindIII - No additional codons in the induced protein

Prom

Xhol

arsR

LucFF

HindIII

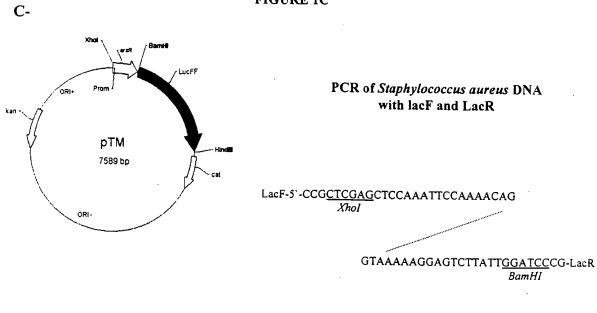
TGAGAAA<u>AGGAGG</u>CGGATCCATG

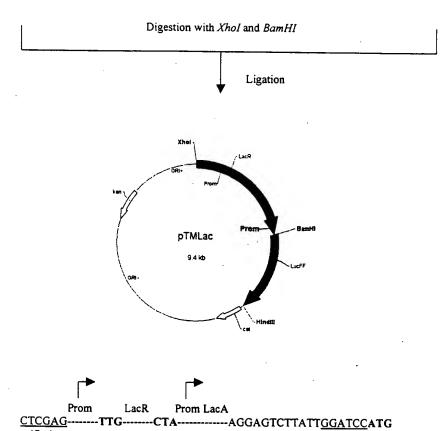
BamHI

RBS

Xhol

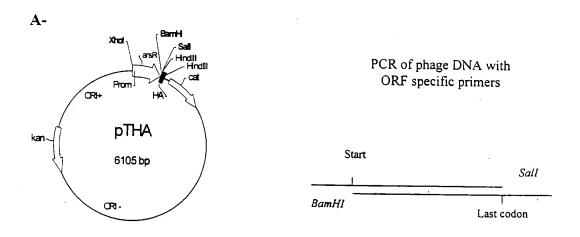
FIGURE 1C

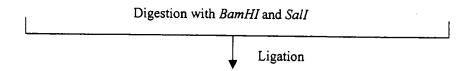


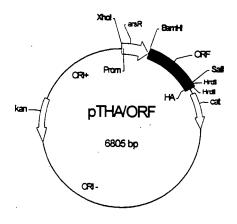


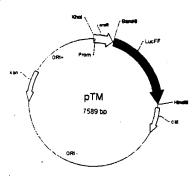
BamHI

FIGURE 2A









PCR of phage DNA with ORF specific primers



Digestion with BamHI and HindIII

Ligation

And Plant OFF

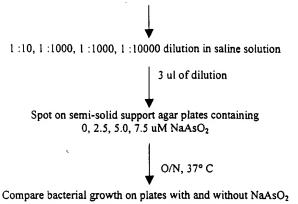
7589 bp

CRI.

DUTETESE DELEGA

A) Functional assay on semi-solid support medium

O/N culture inoculated from frozen stock of phage ORF Staphylococcus aureus RN4220 transformants



B) Functional assay in liquid medium

O/N culture inoculated from frozen stock of phage ORF Staphylococcus aureus RN4220 transformants

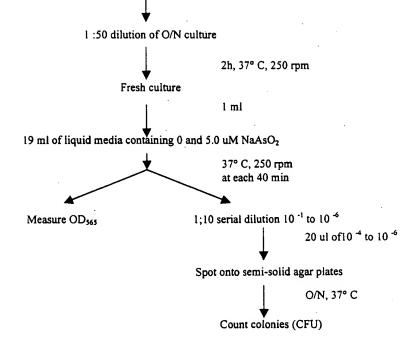


FIGURE 4A

A-

44AHJD ORFs tested for functional analysis

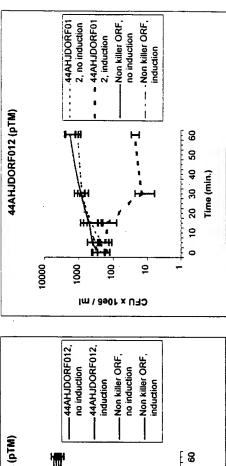
| UID | POS |
|--------------|------------|
| 44AHJDORF001 | 1262710342 |
| 44AHJDORF002 | 37895732 |
| 44AHJDORF003 | 66268389 |
| 44AHJDORF004 | 876410227 |
| 44AHJDORF005 | 1389012643 |
| 44AHJDORF006 | 8032029 |
| 44AHJDORF007 | 20443027 |
| 44AHJDORF008 | 30203775 |
| 44AHJDORF009 | 57446496 |
| 44AHJDORF010 | 1442013938 |
| 44AHJDORF011 | 1559315225 |
| 44AHJDORF012 | 83918813 |
| 44AHJDORF013 | 1499614586 |
| 44AHJDORF019 | 98369630 |
| 44AHJDORF023 | 64946315 |
| 44AHJDORF025 | 1517514999 |
| 44AHJDORF027 | 1291613080 |
| 44AHJDORF028 | 92359071 |
| 44AHJDORF035 | 1395713811 |
| 44AHJDORF036 | 1016510019 |
| 44AHJDORF039 | 17431883 |
| 44AHJDORF040 | 97409877 |
| 44AHJDORF044 | 1291712783 |
| 44AHJDORF046 | 48915019 |
| 44AHJDORF048 | 1534015212 |
| 44AHJDORF049 | 57845909 |
| 44AHJDORF053 | 33483467 |
| 44AHJDORF113 | 199600 |
| 44AHJDORF114 | 1617215870 |
| 44AHJDORF121 | 1636216165 |
| 44AHJDORF123 | 614796 |

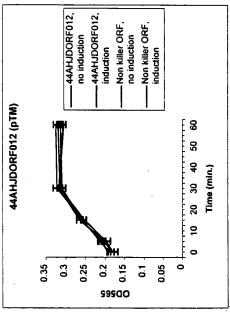
B-

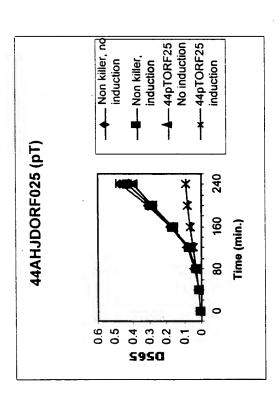
FIGURE 4B

| | | Semi-solid support media | | |
|------------------------------------|---|--------------------------|--|--|
| ORF ID | Staphylococcus aureus transformants | Without induction | With induction (5 uM sodium arsenite) | |
| | Clone1 | • <u>\$</u> • * | | |
| | Clone2 | 0.0 | | |
| | Clone3 | | | |
| 44AHJDORF25 Clone1 Clone2 Clone3 | O_=O | | | |
| | Clone2 | 000 | | |
| | Clone3 | O | | |
| Control 77ORF30 | Clone1 | O O 6 | 0.0.9.3 | |
| | | | | |









-Non killer ORF, no

44AHJDORF025 (pT)

induction

44pTORF025, No induction

-44pTORF025, Induction

240 200

80 Time (min.)

40

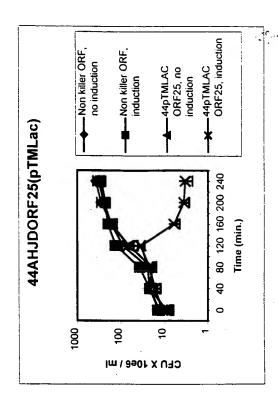
Non killer ORF, induction

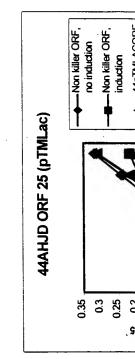
0.1

0.01 0.001 0.0001

CFU X 10e6 / ml

5





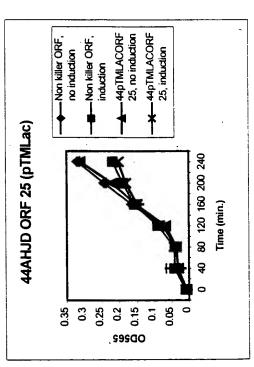


Fig.

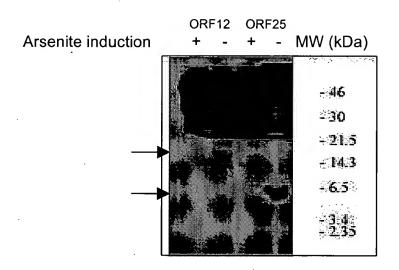
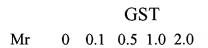


FIGURE 7A



GST ORF25 0 0.1 0.5 1.0 2.0 0 0.1 0.5 1.0 2.0 ACB

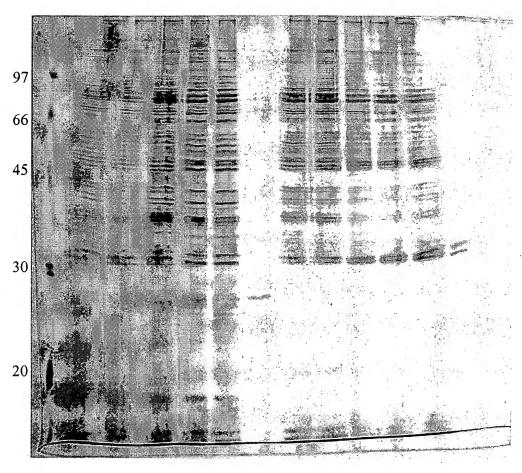


FIGURE 7B

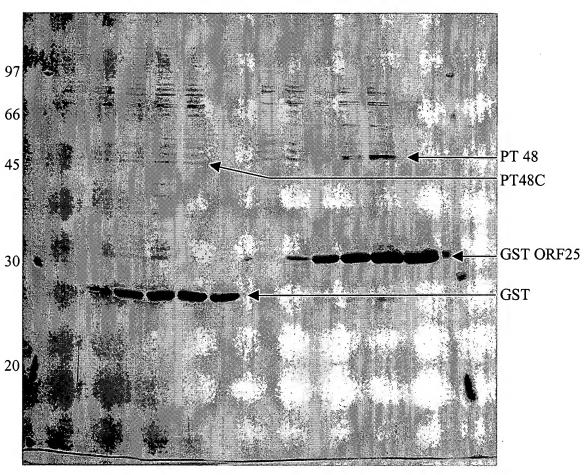
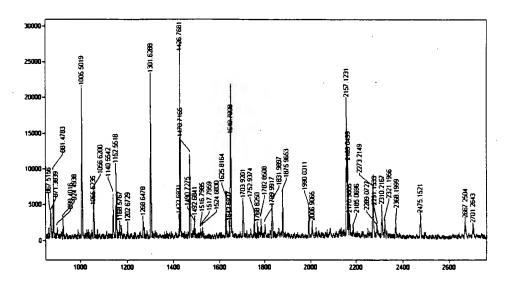


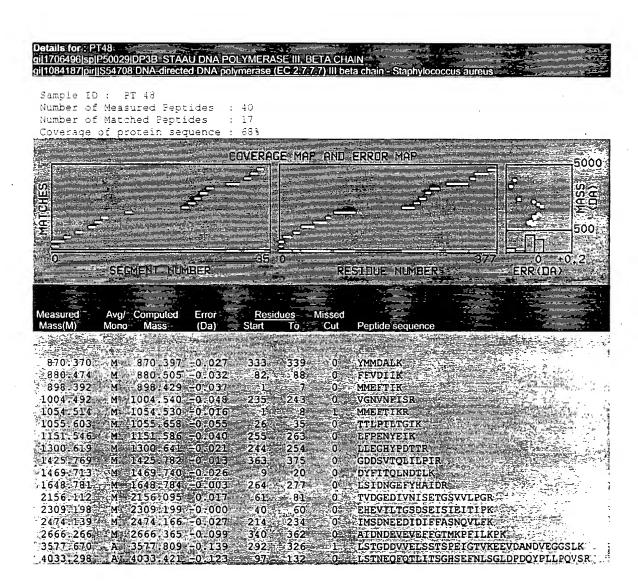
FIGURE 8

Tryptic peptide mass spectrum of 48 kDa interacting protein

(from Figure 1B)



Identification of PT48 as DNA-directed DNA polymerase III beta.



SEQ ID NO: 1 Staphyloccus aureus DnaN nucleotide sequence

ATGATGGAATTCACTATTAAAAGAGATTATTTTATTACACAATTAAATGACACATTAAAA GCTATTTCACCAAGAACAACATTACCTATATTAACTGGTATCAAAATCGATGCGAAAGAA CATGAAGTTATATTAACTGGTTCAGACTCTGAAATTTCAATAGAAATCACTATTCCTAAA ACTGTAGATGGCGAAGATATTGTCAATATTTCAGAAACAGGCTCAGTAGTACTTCCTGGA CGATTCTTTGTTGATATTATAAAAAAATTACCTGGTAAAGATGTTAAATTATCTACAAAT GAACAATTCCAGACATTAATTACATCAGGTCATTCTGAATTTAATTTAAGTGGCTTAGAT CCAGATCAATATCCTTTATTACCTCAAGTTTCTAGAGATGACGCAATTCAATTGTCGGTA AAAGTGCTTAAAAACGTGATTGCACAAACAAATTTTGCAGTGTCCACCTCAGAAACACGC CCAGTACTAACTGGTGTGAACTGGCTTATACAAGAAAATGAATTAATATGCACAGCGACT GACTCACACCGCTTGGCTGTAAGAAAGTTGCAGTTAGAAGATGTTTCTGAAAACAAAAAT GTCATCATTCCAGGTAAGGCTTTAGCTGAATTAAATAAAATTATGTCTGACAATGAAGAA ATTTCTCGATTATTAGAAGGACATTATCCTGATACAACACGTTTATTCCCTGAAAACTAT GAAATTAAATTAAGTATAGACAATGGGGAGTTTTATCATGCGATTGATCGTGCCTCTTTA TTAGCGCGTGAAGGTGGTAATAACGTTATTAAATTAAGTACAGGTGATGACGTTGTTGAA TTGTCTTCTACATCACCAGAAATTGGTACTGTAAAAGAAGAAGTTGATGCAAACGATGTT ATCGATAATGATGAGGTTGAAGTTGAATTCTTCGGTACAATGAAACCATTTATTCTAAAA CCAAAAGGTGACGACTCGGTAACGCAATTAATTTTACCAATCAGAACTTACTAA

SEQ ID NO: 2 Staphyloccus aureus DnaN amino acid sequence

>gi|1084187|pir||S54708 DNA-directed DNA polymerase (EC 2.7.7.7) III beta chain - Staphylococcus aureus

MMEFTIKRDYFITQLNDTLKAISPRTTLPILTGIKIDAKEHEVILTGSDSEISIEITIPKTVDGEDIVNI SETGSVVLPGRFFVDIIKKLPGKDVKLSTNEQFQTLITSGHSEFNLSGLDPDQYPLLPQVSRDDAIQLSV KVLKNVIAQTNFAVSTSETRPVLTGVNWLIQENELICTATDSHRLAVRKLQLEDVSENKNVIIPGKALAE LNKIMSDNEEDIDIFFASNQVLFKVGNVNFISRLLEGHYPDTTRLFPENYEIKLSIDNGEFYHAIDRASL LAREGGNNVIKLSTGDDVVELSSTSPEIGTVKEEVDANDVEGGSLKISFNSKYMMDALKAIDNDEVEVEF FGTMKPFILKPKGDDSVTQLILPIRTY